# Effects of Ginseng Total Saponin on [³H]DAGO Bindings of Opioid μ-Receptors

**Ki-Wan Oh#, Hwa-Kyung Lim\*, Cheol-Beum Park, Im-Chul Shin and Jin-Tae Hong**

*Department of Pharmacology, College of Pharmacy, Chungbuk National University, Cheongju 360-763, Korea*
*\*Korea Food and Drug Administration, Seoul 122-704, Korea*
(Received September 16, 2002)

**Abstract :** Crude synaptic membrane fractions from the frontal cortex, striatum, brain stem and whole brain of rat were prepared to assay the effects of ginseng total saponin (GTS) on [³H]DAGO bindings of the opioid μ-receptors. Scatchard plots analysis binding data demonstrated that GTS (0.1 mg/ml) decreased the affinity of specific [³H]DAGO bindings without changes in $B_{max}$ in the frontal cortex and striatum. On the other hand, GTS did not affect the [³H]DAGO bindings in the brain stem and whole brain. These results suggest that the regulation of [³H]DAGO bindings by GTS may play roles in the change of the pharmacological responses of μ-opioids.

**Key words :** Ginseng total saponin (GTS), [³H]DAGO, μ-opioid, synaptic membrane, receptor bindings

## INTRODUCTION

Ginseng, the root of Panax ginseng C. A. Meyer (*Araliaceae*) is well known as a herbal medicine and has been used in therapy for thousands of years. Many reports have provided evidence that ginseng has a variety of effects on the central nervous systems (CNS). Ginseng not only has stimulative and inhibitive effects on the CNS,[1-4] but also regulates the effects of sedatives, hypnotics and convulsants.[5] Panax ginseng also acts a modulator of neurotransmitters in the brain.[6,7]

On the other hand, it has been reported that ginseng extract and ginseng saponin antagonizes morphine-induced analgesia, locomotion and catalepsy.[8-12] Meanwhile, it has been suggested that these antagonisms of morphine effects by ginseng might be mediated by indirect activity on dopaminergic system. In term of this hypothesis, some evidences provided that ginseng saponin inhibited cocaine- and methamphetamine-induced hyperactivity in mice. Moreover, it was reported that ginseng inhibited the dopamine receptor supersensitivity induced by cocaine and methamphetamine.[13-15]

However, we could not exclude the possibility of direct action of ginseng total saponin on opioid receptors. Espe-

cially, antagonism of morphine induced-analgesia by ginseng total saponin might be mediated by direct regulation of opioid μ-receptors. Therefore, this study was undertaken to investigate the interactions of ginseng total saponin with [³H]DAGO bindings on opioid μ-receptors.

## MATERIALS AND METHODS

### 1. Materials

{[³H]*d*-Ala², *N*-Me⁴, Gly⁵-ol}-enkephalin {[³H]DAGO} was purchased from New England Nuclear (Boston, Mass., USA). DAGO was obtained from Research Biochemicals Inc. (Natrick, Mass., USA.). Ginseng total saponin fraction [saponins mixture containing at least 10 glycosides known as ginsenosides from Panax ginseng C. A. Meyer, extracted and purified by Namba *et al.*'s method[16]] was supplied by the Korea Ginseng and Tobacco Research Institute. All other chemicals used receptor binding studies were obtained from Sigma Chemical Co. (St. Louis, MO., USA.).

### 2. Animals

Male Sprague-Dawley rats (Charles River, Wilmington, Mass.) weighing 200-250 g were maintained at constant temperature (22-24°C), 4 to a cage. The animals were allowed free access to food and water in a room automatically illuminated for 14 hr and kept dark for 10 hr.

#To whom correspondence should be addressed.
(Tel) +82-43-261-2827; (Fax) +82-43-268-2732
(E-mail) kiwan@trut.chungbuk.ac.kr

## 3. Membrane preparation

The rats were decapitated and their brains were removed, and whole brain, frontal cortex, striatum, brain stem dissected on ice according to the methods described by Glowinski and Iversen,[17] and Segal and Kuczenski.[18] Crude synaptic membrane fractions were prepared following the method of Zukin et al.,[19] with slight modification[20]). Whole brain, frontal cortex, striatum, brain stem were homogenized in 10 volume of ice-cold Tris-HCl buffer (pH 7.4) with a Polytron. The homogenate was centrifuged at 48,000 g for 10 min at 4°C. The supernatant was discarded and then pellet were re-suspended in membrane buffer, homogenized, centrifuged as before. After the second centrifugation, the supernatant was discarded, and the pellet was re-suspended in ice assay buffer. Then, the membrane was assayed for protein content,[21] and aliquots were stored at 80°C.

## 4. Binding assays

The frozen pellet was thawed, suspended in 50 mM Tris-citrate buffer and centrifuged at 48,000 g for 20 min.

The pellet obtained was re-suspended with 40 volumes of 50 mM Tris-citrate buffer and incubated at 25°C for 30 min to remove endogenous inhibitors. After centrifugation at 48,000 g for 20 min, the pellet was then suspended in the buffer for binding. The membrane homogenates was incubated at 25°C for 1 hr in 50 mM Tris-HCl buffer (pH 7.4) with 1 nM [3H]DAGO (specific activity, 40.8 Ci/mmol) in a total volume 1 ml. The reaction was terminated using a Brandel cell harvester (Model M-24, Brandel, MD, USA.) and the samples were filtered through Whatman GF/B glass filters pre-soaked in 50 mM Tris-HCl buffer at 4°C for 2 hr. Filters were washed three times with 5 ml of 50 mM Tris-HCl buffer (pH 7.4, 4°C) and were transferred to scintillation counting vials containing 0.5 ml of a tissue solubilizer (Solubene-350, Packard Instrument Company, Meriden, CT, USA) and 4 ml of a scintillation cock-tail (Hionic Fluor, Packard Instrument Company). Non-specific binding was measured in the presence of 10 mM unlabelled DAGO. Comparable results were obtained from at least three independent sets of experiments.
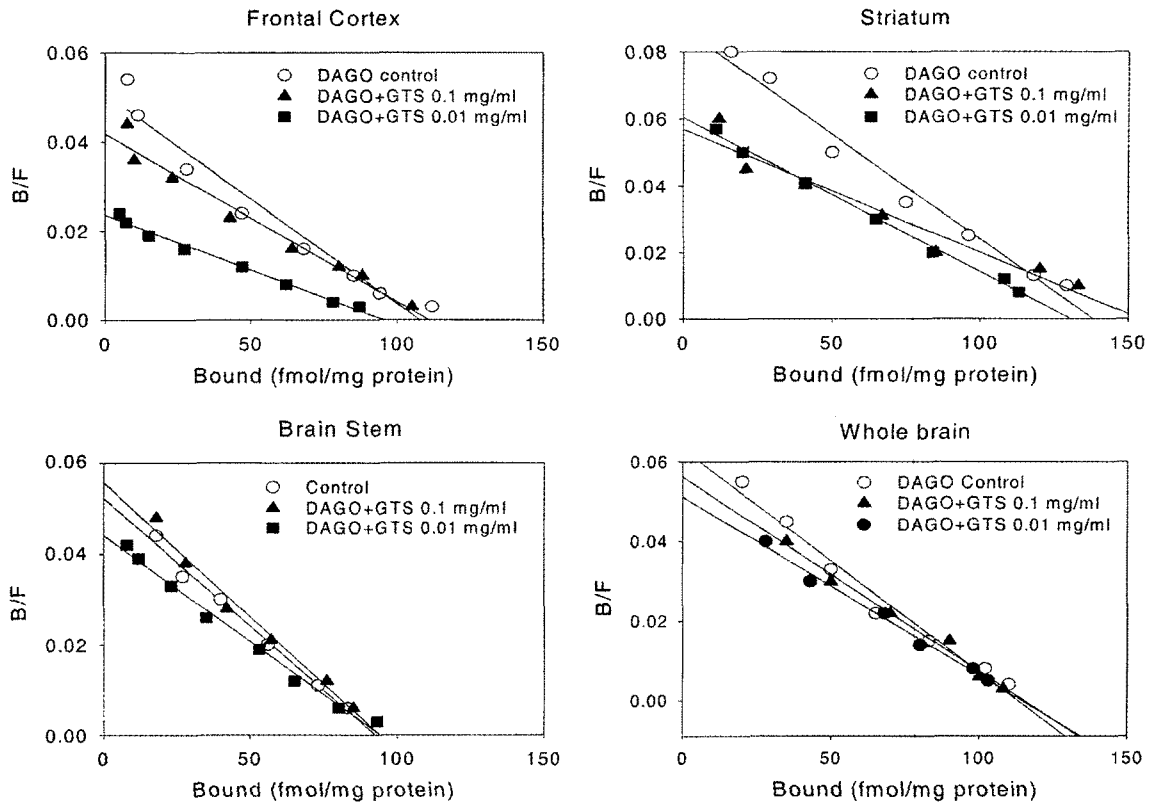


Fig. 1. Representative Scatchard plots of [3H]DAGO bindings and the effects of ginseng total saponin. Crude synaptic membranes prepared from frontal cortex, striatum, brain stem and whole brain were assayed for [3H]DAGO bindings (0.1 nM) in the presence of ginseng total saponin (0.1 and 0.01 mg/ml). Non-specific binding was determined in the presence of 1 μM of DAGO.

**Table 1.** Effects of GTS on specific [³H]DAGO bindings

| | Concentration | Kd (nM) | Bmax (fmol/mg protein) |
|---|---|---|---|
| Frontal cortex | Control | 1.65±0.28 | 113.1±5.7 |
| | GTS 0.1 mg/ml | 2.75±0.33* | 104.2±4.7 |
| | 0.01 mg/ml | 1.30±0.18 | 112.5±4.5 |
| Striatum | Control | 0.84±0.09 | 131.6±6.3 |
| | GTS 0.1 mg/ml | 1.25±0.19* | 149.7±7.1 |
| | 0.01 mg/ml | 1.07±0.10 | 143.3±6.9 |
| Brain stem | Control | 1.25±0.12 | 104.1±4.4 |
| | GTS 0.1 mg/ml | 1.58±0.27 | 98.6±3.1 |
| | 0.01 mg/ml | 1.24±0.18 | 102.3±5.8 |
| Whole brain | Control | 1.25±0.14 | 115.6±4.9 |
| | GTS 0.1 mg/ml | 1.38±0.18 | 112.3±5.6 |
| | 0.01 mg/ml | 1.24±0.15 | 116.8±5.0 |

Each value represented the mean ± SEM. Each experiment was performed in triplicated.
*P<0.05 compared with that of control.

## RESULTS

[³H]DAGO produced curvilinear Scatchard plots which could be fitted to a one-binding site model, yielding a straight line (Fig. 1). Scatchard analysis of binding data demonstrated that GTS (0.1 mg/ml) decreased the affinity of specific [³H]DAGO bindings without changes in $B_{max}$ in the frontal cortex and striatum. However, GTS did not affect the [³H]DAGO bindings in the brain stem and whole brain (Table 1).

## DISCUSSION

In the present study, we found that GTS (0.1 mg/ml) decreased the affinity of specific [³H]DAGO bindings without changes in $B_{max}$ in the frontal cortex and striatum. Therefore, we suggest that GTS could modulate the opioid μ-receptor bindings to synaptic membrane of the frontal cortex and striatum of rat. However, lower concentration of GTS did not affect the specific [³H]DAGO bindings in the frontal cortex and striatum. Moreover, GTS also did not affect the specific [³H]DAGO bindings to the synaptic membrane in other brain regions such as brain stem and whole brain.

Ginseng modulated morphine-induced behaviors that seemed to be related to the opioid receptors. Morphine indirectly stimulates dopaminergic systems. In particular, the μ-receptor site may be critical for the effects of opioids on the dopaminergic systems. GTS inhibited morphine-induced hyperactivity, the development of reverse tolerance to ambulation-accelerating effect of morphine.[11] Recently, GTS also inhibited the conditioned place preference and the post-synaptic dopamine receptor supersensitivity induced by morphine.[11,12] Therefore, it is suggested that GTS might be able to modualte the dopaminergic systems. So, we previously reported that GTS might be useful for the prevention of opioids-induced toxicity.

On the other hand, GTS antagonized morphine-induced analgesia, inhibited the development of analgesic tolerance to and the physical dependence on morphine.[10] In term of these effects, we suggest that GTS directly acts on opioid receptors. Thus, this study was undertaken to understand the interactions of GTS with [³H]DAGO bindings. From this study, we confirmed that GTS decreased the affinity of specific [³H]DAGO bindings at some brain regions especially, frontal cortex and striatum. Because GTS showed the slightly increased affinity of specific [³H]DAGO bindings at the low dose, even though it is not significant.

There is another evidence that GTS and ginsenosides act on other pharmacological receptor sites. GTS inhibited $GABA_A$ and $GABA_B$ receptor bindings to the synaptic membrane of frontal cortex.[22] Therefore, GTS also plays important roles in the regulation of GABAergic neurotransmission.

Ginseng exhibited various pharmacological activities on the CNS. These effects mean that ginseng has the affinity to more than one receptors type on the CNS. From this study, we suggest that ginseng saponin could inhibit on opioid μ-receptors bindings in some regions of the brain. In conclusion, Ginseng saponin can modulate the morphine-induced pharmacological effects through opioid μ-receptors.

Further research requires what kind of ginsenosides have the binding affinity to opioid μ-receptors.

## REFERENCES

1. Petkov, V. W. : Pharmacological investigation of the drug Panax ginseng C. A. Meyer. Arzneim. Forsch. (Drug Res.). **9**, 305-311 (1939).
2. Takagi, K., Saito, H. and Nabata, H. : Pharmacological studies of panax ginseng root: estimation of pharmacological actions of panax ginseng root. Jpn. J. Pharmac. **22**, 245-259 (1972).
3. Saito, H., Tsuchiya, M., Nata, S. and Takagi, K. : Effects of panax ginseng root on the acquisition of second discrimination behavior in rats. Jpn. J. Pharmacol. **27**, 509-616 (1977).

4. Saito, H., Tsuchiya, M., Nata, S. and Takagi, K. : Effects of panax ginseng root on conditional avoidance response in rat. Jpn. J. Pharmacol. **29**, 310-325 (1977).

5. Oh, J. S., Park, C. W. and Moon, D. Y. : Effects of panax ginseng on the central nervous system. Kor. J. Pharmacol. **5**, 23-28 (1969).

6. Tsang, D., Yeung, H. W., Tso, W. W., Peck, H. and Lay, L. P. : Effect of saponins isolated from ginseng on the uptake of neurotransmitter in rat brain synaptosomes. Neurosciences Letter (supp.) **12**, S20 (1983).

7. Kim, Y. C., Lee, J. H., Kim, M. S. and Lee, N. G. : Effect of the saponin fraction of panax ginseng on catecholamines in mouse brain. Arch. Pharm. Res. **8**, 45-39 (1985).

8. Kim, H. S., Jang, C. G. and Lee, M. K. : Antinarcotic effects of standardized ginseng G115 on morphine, Planta Medica **56**, 158-163 (1990).

9. Ramarao, P. and Bhargava, H. N. : Antagonism of the acute pharmacological actions of morphine by Panax ginseng extract, Gen. Pharmacol. **21**, 877-880 (1990).

10. Kim, H. S., Oh, K. W., Oh, S., Rheu, H. M. and Seong, Y. H. : Antagonism of morphine analgesia by the pretreatment sites with ginseng total saponin, Kor. J. Ginseng Sci. **15**(1), 1-6 (1991).

11. Kim, H. S., Jin-Goo Kang, and Oh, K. W. : Inhibition by ginseng total saponin of the development of morphine reverse tolerance and dopamine receptor supersensitivity in mice, Gen. Pharmacol. **26**(5), 1071-1076 (1995).

12. Kim, H. S., Jang, C. G., Oh, K. W., Oh, S., Rheu, H. M., Rhee, G. S., Seong, Y. H. and Park, W. K. : Effects of ginseng total saponin on morphine-induced hyperactivity and conditioned place preference in mice, J. Ethnopharamcol. **60**, 33-42 (1998).

13. Kim, H. S., Jang, C. G., Seong, Y. H., Nam, K. Y. and Oh, K. W. : Blockade by ginseng total saponin pf the development of cocaine-induced reverse tolerance and dopamine

receptor supersensitivity in mice. Pharmacol. Biochem. Behav. **50**, 23-27 (1995).

14. Kim, H. S., Jang, C. G., Rheu, H. M., Cho, D.H. and Oh, K. W. : Blockade by ginseng total saponin pf the development of methmaphetamine reverse tolerance and dopamine receptor supersensitivity in mice. Planta Medica **61**, 22-25 (1995).

15. Kim, H. S., Jang, C. G., Oh, K. W., Seong, Y. H., Rheu, H. M., Cho, D.H. and Kang, S. Y. : Effects of ginseng total saponin on cocaine-induced hyperactivity and conditional place preference in mice Pharmacol. Biochem. Behav. **53**, 185-190 (1996).

16. Namba, T., Yoshizaki, T., Tominori, K., Kobashi, K., Mitsui, K. and Hasse, J. : Fundamental studies on the evaluation of the crude drugs (1), Planta Med. **32**, 588-394 (1974).

17. Glowinski, J. and Iversen, L. L. : Regional studies of catecholamines in the brian: The disposition of $[^3H]$norepinephrine, $[^3H]$dopanine and $[^3H]$DOPA in various regions of the brain. J. Neurochem. **13**, 655-669 (1966).

18. Segal, D. S. and Kuczenski, R. : Tyrosine hydroxylase activity; regional and subcellular distribution in brain. Brain Res. **68**, 261-266 (1974).

19. Zukin, S. R., Young, A. B. and Snyder, S. H. : Gamma aminobutyric acid binding to receptor sites in the rat central nervous system. Proc. Natn. Acad. Sci. USA. **71**, 4802-4807 (1974).

20. Ito, Y. and Kuriyama, K. : Some properties of solubilized GABA receptor. Brain Res. **236**, 351-363 (1982).

21. Lowery, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. : Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**, 265-275 (1951).

22. Kimura, T., Saunders, P. A., Kim, H. S., Rheu, H. M., Oh, K. W. and Ho, I. K. : Interactions of ginsenosides with ligand-bindings of $GABA_A$ and $GABA_B$ receptors. Gen. Pharmacol. **25**(1), 193-199 (1994).